On the Mechanism of Peptidoglycan Binding and Cleavage by the endo-Specific Lytic Transglycosylase MltE from Escherichia coli

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Published in:
Biochemistry

DOI:
10.1021/bi300900t

Publication date:
2012

Supplementary Info

Fibriansah *et al.* (2012) On the mechanism of PG binding and cleavage by the endo-specific lytic transglycosylase MltE from *Escherichia coli*

Table S1. Se-MAD Data Collection and Phasing Statistics

		SeMet-sMltE	
Data collection	inflection	peak	remote
Beamline		BM14 (ESRF)	
Wavelength (Å)	0.9788	0.9790	0.8855
Space group		$P2_12_12_1$	
Unit cell dimensions		a = 77.6 Å b = 94.6 Å c = 160.8 Å	
Max. resolution (Å)	2.75	2.75	2.75
No. of measured reflections	96998	95894	96424
No. of unique reflections	28972	28898	28853
Completeness (%)	92.8	92.3	92.2
R_{sym}	0.055	0.061	0.065
Mean I/s	9.4	8.3	8.0
MAD phasing	figures of merit (centric/acentric): 0.53/0.57		

Figure S1

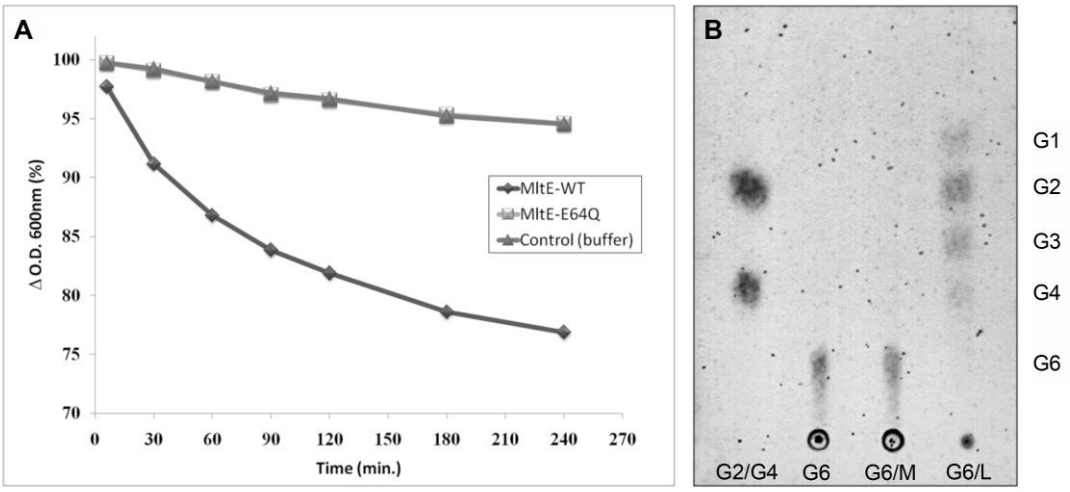

Figure S1. Peptidoglycanolytic and chitinolytic assays. (A). Turbidimetric assay of PG solubilization by sMltE. The decrease in turbidity (measured at 600 nm) of insoluble *M. luteus* cell wall (in 50 mM MES pH 6.0 and 200 mM NaCl) was monitored with time after addition of (♦) sMltE-WT, (■) sMltE-E64Q and (▲) buffer only (control). The turbidity decrease data is presented as the percentage decrease from the initial value (using an interpolated OD_{600} at t = 0 min), and calculated with the averaged data of triplicate experiments. (B) The chitinolytic activity of MltE was assayed by analysing the products resulting from a reaction with chitohexaose as substrate using TLC. Hen egg-white lysozyme was used as positive control. M: sMltE; L: lysozyme; G1: GlcNAc; G2: $(GlcNAc)_2$; G3: $(GlcNAc)_3$; G4: $(GlcNAc)_4$; G6: $(GlcNAc)_6$.

Supplementary Figure S2

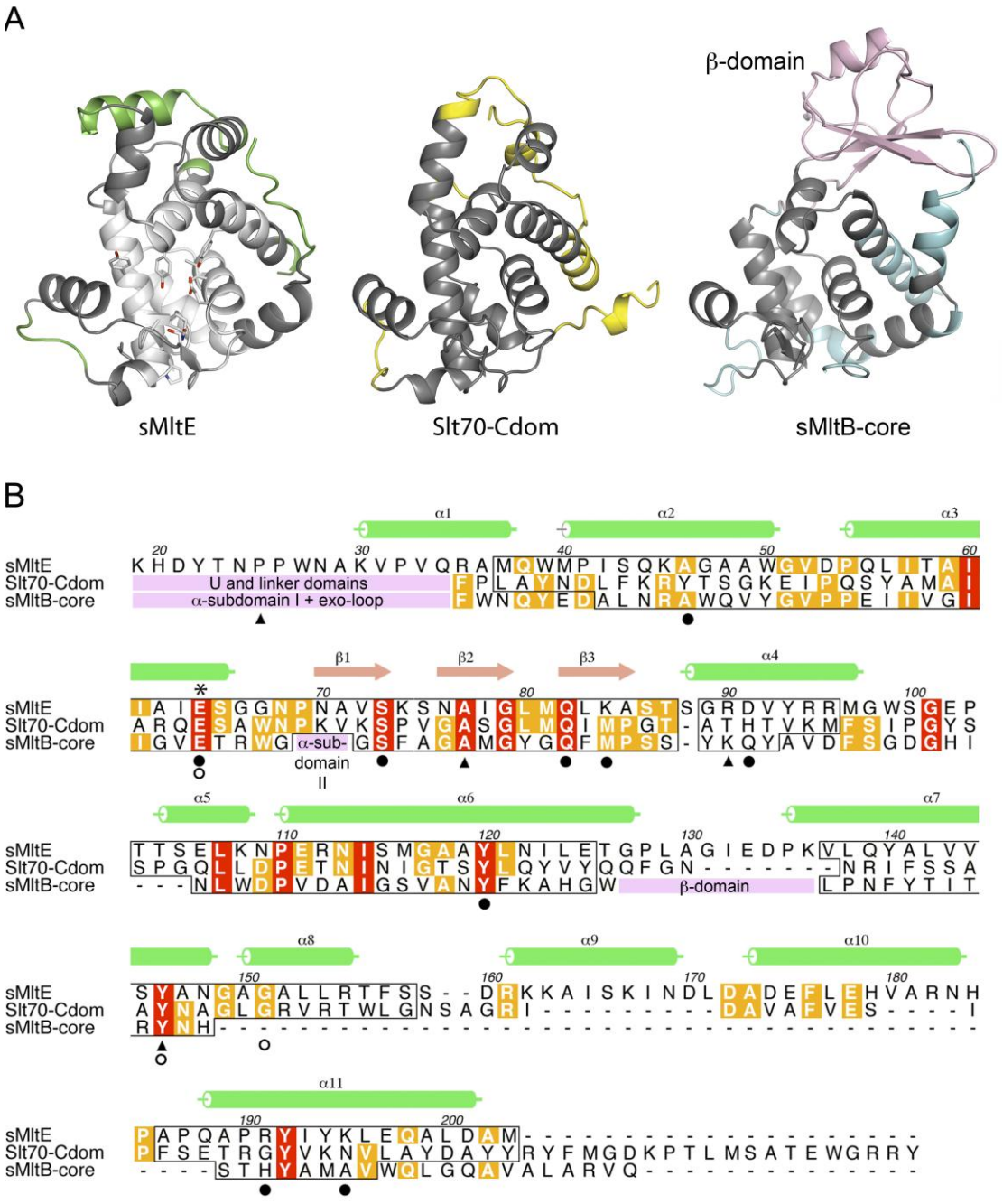

Figure S2. Comparison of sMltE with the LT-domains of Slt70 and sMltB. (A) Overall comparison of the Cα backbone structures (ribbon representation) of the three proteins. Unique segments in sMltE, Slt70-Cdom (LT domain of Slt70) and sMltB-core (LT domain of MltB) with no equivalents in the other proteins are colored green, yellow and cyan, respectively. The unique β-domain in MltB is colored pink. Segments in sMltE that have an equivalent in either one or both of the other LTs are colored dark-grey and light-grey, respectively. Segments in Slt70-Cdom and sMltB-core that have an equivalent in sMltE are colored dark-grey. Residues that are identical in all three proteins are shown in stick representation. (B) Structure-based sequence alignment of the three *E. coli* proteins. The amino acid residue numbering and secondary structure labeling is based on MltE. Common core residues are enclosed in boxes. Residues that are identical in two or three proteins are shown as white letters on an orange or a red background, respectively. The catalytic acid/base is indicated with an asterisk. Spheres below the sMltB sequence indicate the residues in sMltE with a role in saccharide binding.

Supplementary Figure S3

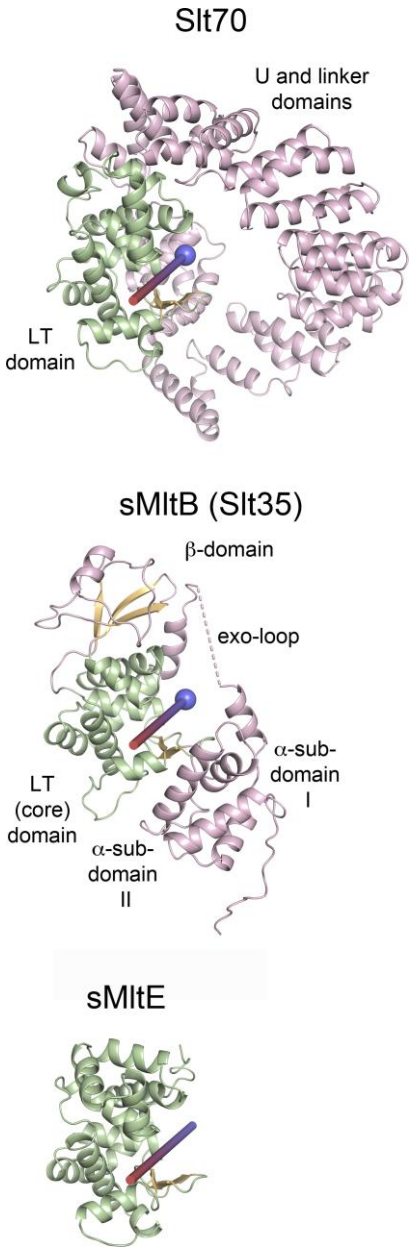

Figure S3 Structural basis for the exo- or endo-specific cleavage specificity of Slt70, MltB and MltE. The overall structures of the three proteins are shown in ribbon representation with the LT domains colored in green and the non-LT domains in pink. A bound glycan chain is shown schematically as a solid cylinder (colored with a red to blue gradient), with a terminal 1,6-anhydroMurNAc residue represented by a blue ball. The U and linker domains in Slt70 and the exo-loop in sMltB present a steric obstruction that limit the accessibility of the PG-binding groove, explaining their exo-lytic activity. The absence of such an obstruction in sMltE explains its endo-lytic activity.

Supplementary Figure S4

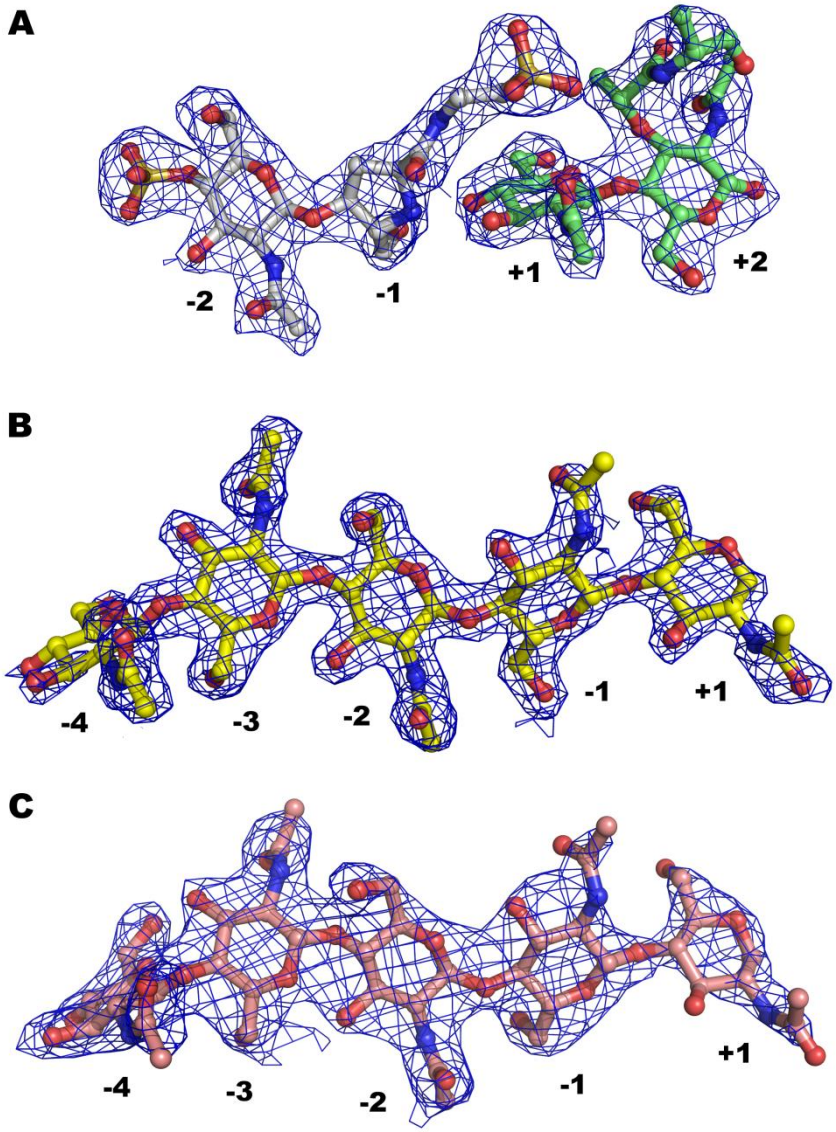

Figure S4. The $2F_o$-F_c electron density maps for the bound ligands are shown, contoured at 1σ. (A) Bulgecin A and the murodipeptide GlcNAc-MurNAc-L-Ala-D-Glu in protein molecule A of the asymmetric unit of sMltE/bulgA•GM-Ala-Glu. (B-C) Chitopentaose bound to protein molecule A in the 1.9 Å and 2.4 Å sMltE-E64Q/G5 structure, respectively.

Supplementary Figure S5

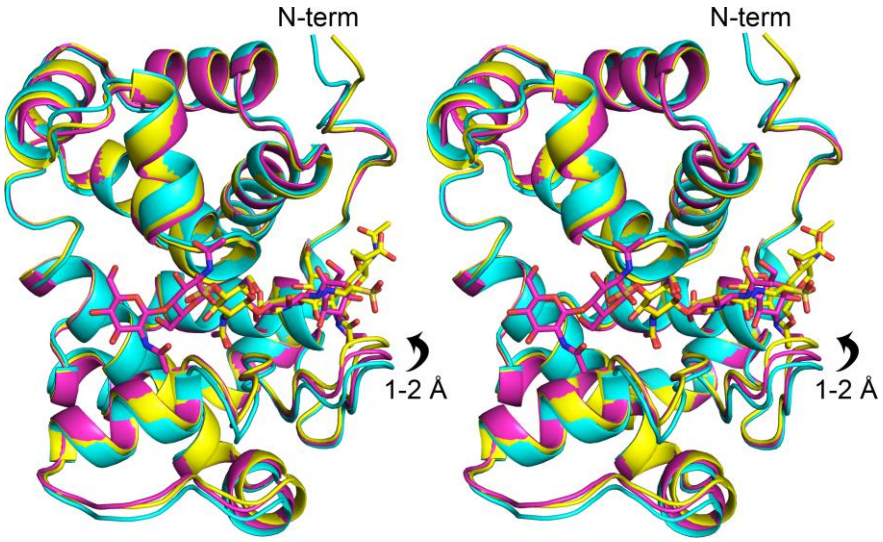

Figure S5. Stereo diagram showing an overlay of the apo structure of sMltE (cyan) with the structures of sMltE-E64Q/G5 (purple) and sMltE/bulgA•GM-Ala-Glu (yellow).